DNA Supramolecular Complexes : Structural and Functional Peculiarities as Studied by Scanning Atomic Force Microscopy

A.S.Elkady[1,4,*], A.Alexeev[2], Y.Sebyakin[3], M.Gallyamov[1], A.Moskovotsov[4], G.Bischoff[5], , R.Zhdanov[4] and A.R.Khokhlov[1]

[1] Physics Department, Moscow State University, Moscow 117234, Russia
[2] NT-MDT, State Research Institute of Physical Problems, 103460, Moscow,Russia
[3] M.V.Lomonosov academy of Fine Chemical Technology, Moscow, Russia
[4] V.N.Orekhovich Institute of Biomedical Chemistry, Russian academy of Medical Sciences, 10, Pogodinskaya st., Moscow 119992, Russia
[5] Institute of Biochemistry, Martin-Luther University, Kurt-Mothes Str.3, Halle/Saale 06120, Germany
*** Author to whom correspondence: e-mail: ashraf_elkady@yahoo.com, Fax: 7(095)9328820**

DNA is a remarkable polymer that exhibits a complex phase behavior as a function of packing density, salt concentration, and other variables [1,2]. When mixed with cationic liposomes (CL), complexes known as "lipoplexes"- are formed in which the cationic lipids replace the role of the positive counterions. These DNA-lipid complexes are potential vectors for injecting DNA into cells and play an important role in the emerging field of non-viral gene therapy [3-5]. In order to develop an optimal cationic gene carrier, and predict what aspects to modify the target for interaction with synthetic or therapeutic molecules, more knowledge regarding interactions and structures of DNA-CL complexes is required. In the present study Atomic Force Microscopy (AFM) was applied to elucidate the structural peculiarities for a series of DNA-lipid complexes and identify the key parameters that are crucial for optimising gene transfer by correlating the structural features to transfection efficiencies.

Cationic liposomes were prepared from a novel dicationic lipid, with the chemical formula given in Fig.1, using lipid hydration method for different lipid derivatives [6]. The liposomes prepared when n= 2 and m=4 showed high efficiency, when they were complexed with plasmid DNA and used for cell transfection (data not shown). The morphology of such

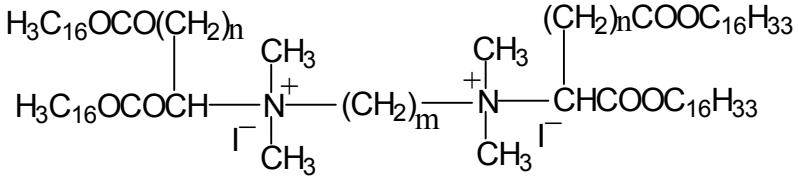

Fig.1. The chemical formula for the synthesized cationic lipid, n=1 (Aspiragine), m=3,4,7,11,22; n=2 (Glutamine)

complexes was studied using AFM which revealed a compact toroid-like structure for such DNA-CL complex (see Fig.2). This compact and moderate size of these condensates may justify their superior transfection efficiency over the other derivatives (where m=3, 7,11, 22).

Fig.2.The cationic liposomes- pDNA complexes (a) AFM image for the complex taken after adhesion to the mica surface.(b),(c) Are diameter and height distributions respectively for the complexes shown in (a)

Felgner et al. [7,8] originally proposed a *"bead-on-string" structure* of the DNA-CL complexes. In the present work, it was interesting to monitor a similar structure, using AFM, for pDNA complexed with a neutral lipid containing calcium gluconate and calcium levulinate (Ca-lip-D, MISR CO.FOR PHARM.IND., Cairo, Egypt). Fig.3 represents the AFM images for such complexes at different pDNA and lipid concentrations.

Fig.3. AFM images for pDNA complexed with neutral lipid through metallic cation bridges (a) the lipid molecules decorating a network of pDNA at high DNA concentration. (b) The lipid molecules surrounding and attached to linearized pDNA molecule in a bead on string regime. (c) Circular pDNA molecules sandwiched by the lipid matrix at a high lipid concentration. (d) Lamellar organization of the lipid molecules along with huge DNA-lipid aggregates.

We reported before a new approach to lipofection, using non-cationic liposomes [9]. The present study may also raise the quesion about the feasibility of using such simple lipids as gene carriers, as their cost is much lower than the commercially available cationic liposomes. Besides, they are more safe and of lower toxicity in vivo.

It was demonstrated in experiments with synthetic DNA polynucleotides that neutral lipids- oleic acid and cholesterol- are tightly bound to DNA double helix, oleic acid being located in the minor groove and cholesterol intercalated between nucleic acid basis [10]. In the present study, AFM was used to assess the nucleic acid affinity to oleic acid. The experiment was carried out with double and trible stranded oligo- and polynucleotides. Fig.4. represents AFM images for such complex before and after ethanol dialysis. Whereas duplexes are influenced

Fig.4. (a) Highly concentrated poly[d(A-T)] without ligand (b) highly concentrated poly[d(A-T)] complexed with oleic acid: 1per 2 base pairs (c) low concentrated poly[d(A-T)] complexed with oleic acid after dialysis.

by oleic acid ligandation, which could not be removed by dialysis, no binding occurs to triple stranded DNA.

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